INTER-SPECIES COMPARISON OF MICROSOMAL REDUCTIVE TRANSFORMATION OF BIOLOGICALLY ACTIVE BENFLURON N-OXIDE

L. Skálová*, M. Nobilis[1], B. Szotáková, V. Wsól and E. Kvasnicková

Department of Biochemical Sciences, Faculty of Pharmacy, Charles University
and [1]Institute of Experimental Biopharmaceutics of the Academy of Sciences of the Czech Republic, Hradec Králové, Czech Republic

SUMMARY

Benfluron N-oxide is an anti-neoplastic active metabolite of benfluron (B) /1/. It is generated by flavine-monooxygenase-catalysed reactions /2/ and immediately undergoes subsequent metabolic transformations, the most important of which are reductive reactions /3/. The products of reductive pathways catalysed by two different microsomal enzymatic systems are the tertiary amine benfluron (i.e. the original parent compound) and/or 7-dihydrobenfluron N-oxide. Our studies on the reductive transformation of B N-oxide in rat, mouse, guinea-pig, rabbit, mini-pig and human microsomes have revealed significant species differences both in the yields of respective reduced metabolites and in the conditions essential for the activity of the reductases involved. While B, the original tertiary amine, is the main product of aerobic incubation of B N-oxide with NADPH in rat, mouse and mini-pig, significantly higher activities of the enzymes catalysing the formation of 7-dihydro-B N-oxide have been detected in rabbit and human microsomes. In rat, mouse and mini-pig, NADPH rather than

*Address for correspondence:
L. Skálová
Department of Biochemical Sciences
Faculty of Pharmacy
Charles University
Heyrovskeho 1203
CZ-500 05 Hradec Králové
Czech Republic

NADH is the preferred coenzyme for B formation, and NADPH is also the preferred coenzyme for the formation of 7-dihydro-B N-oxide in most of the species used. The yield of tertiary amine B is higher in anaerobic rather than aerobic conditions in most experimental species studied. Aerobic or anaerobic incubating conditions have an insignificant effect on the formation of 7-dihydro-B N-oxide. Based on the inhibitory effect of CO on the reductive transformation of B N-oxide, cytochromes P450 can be assumed to participate in the formation of B both in rat and mini-pig, and, in mini-pig only, also in the formation of 7-dihydro-B N-oxide. Inter-species comparison of the properties of the reductases participating in the transformation of B N-oxide shows that the rabbit is a suitable model to study reductive transformation of B N-oxide in man.

KEY WORDS

cytochrome P450 reductase activity, N-oxides, microsomal reduction, benfluron N-oxide, cytostatic drugs

INTRODUCTION

The N-oxide of benfluron (5-(2-dimethylaminoethoxy)-7-oxo-7*H*-benzo[*c*]fluorene N-oxide) is one of the main metabolites of benfluron, a potential cytostatic /1,2,4/. Studies on the biological properties of benfluron metabolites and derivatives have shown that B N-oxide displays significant antineoplastic activity both *in vitro* (inhibition of DNA and protein synthesis in Yoshida tumour cells) and *in vivo* (reduced tumour mass and improved survival period of animals after oral administration of B N-oxide to mice with Ehrlich or KH_2 tumours and to DBA_2 mice with LH 1210 leukaemia) /5/.

In vivo and *in vitro* studies on B N-oxide metabolism carried out in rats have revealed that the most significant biotransformation pathways of the substrate are the reductive pathways (Fig. 1). The main metabolite of B N-oxide is the parent tertiary amine benfluron generated by the reduction of the substrate (reduction I). *In vitro*, this reaction takes place in microsomes with NADPH under both aerobic and anaerobic conditions. Results of inhibition studies suggest that cytochrome P450 is involved in reduction I /3/. An additional biotransformation pathway (reduction II), of minor importance in the rat, is the reduction of the 7-

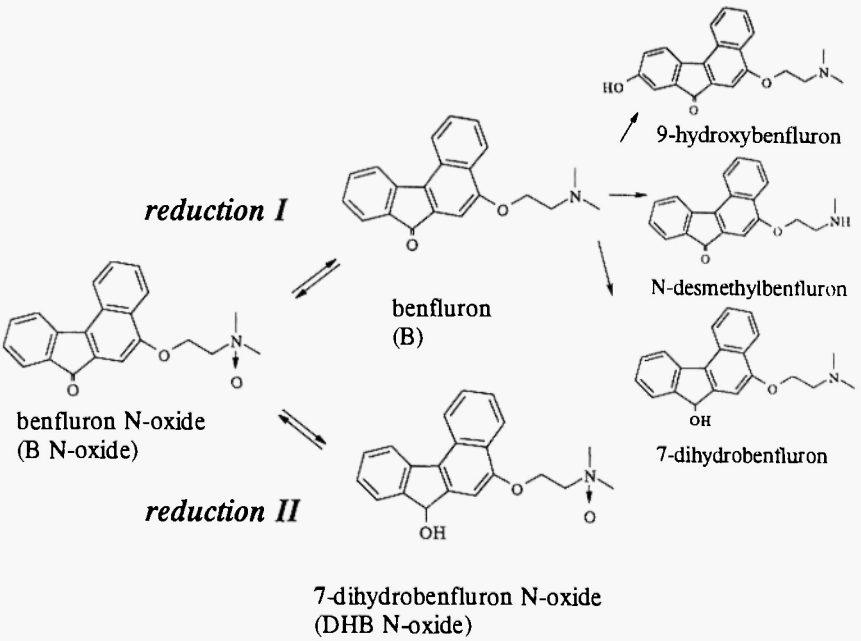

Fig. 1: Microsomal biotransformation of B N-oxide - main metabolic pathways.

oxo group of B N-oxide which gives rise to the N-oxide of 7-dihydro-benfluron (DHB N-oxide) (Fig. 1).

Compared to oxidative biotransformations, the mechanisms of such reductive processes and the enzymes involved have so far been studied to a considerably lesser extent. However, reductive processes play a crucial role in the metabolism of a number of xenobiotics. Reports in the literature show that reductive transformations may have significant detoxification and/or deactivation effects (e.g. in the case of ketones and aldehydes) /6/, or - on the other hand - activation (e.g. naloxan, warfarin, oxisuran) or toxicity-conferring (e.g. azo compounds or quinones) effects /7/. The reduction of B N-oxide to B (reduction I) and the reoxidation of B to B N-oxide represent mutual inter-conversion of two compounds with similar biological activities but with partly different physico-chemical properties and, thus, with likely differences in their respective pharmacokinetic behaviour. The

reduction reaction II which changes the original planar shape of the molecule can be assumed to cause deactivation. The ratio between the reduction reactions I and II therefore represents the ratio of the yields of biologically active and inactive metabolites of B N-oxide.

Our current knowledge of the reductive transformation of B N-oxide is based on experiments carried out in rats. The main problem in using data obtained from experiments on laboratory animals in order to identify biotransformation processes taking place in man is significant inter-species differences in the levels of expression of the genes encoding the enzymes involved in the biotransformation processes and differences in substrate specificity of the enzymes due to (sometimes very minor) changes in the sequence of amino acids in the primary structure of the respective enzymes /8/. Carrying out experiments in several species improves the chance of finding the optimum model to study a specific problem /9/. Inter-species comparisons help us to select the most suitable animal species from the aspect of predicting as precisely as possible metabolic transformation of a specific drug in man. Moreover, the identification of such a suitable species is also crucial for obtaining those metabolites which are otherwise difficult or impossible to be synthesised in quantities that would allow for their identification and, primarily, for testing their biological effects. Inter-species comparison of the activities of biotransformation enzymes may also contribute to our better understanding of the mechanisms of their catalytic effects.

These experiments were aimed at studying the reductive transformation of B N-oxide *in vitro* using microsomes isolated from rat, mouse, guinea-pig, rabbit, mini-pig and human liver homogenates with the goal of comparing the activities and properties of the enzymes catalysing the reductive biotransformation of B N-oxide in microsomes from various laboratory animals and of identifying those species in which the microsomal reductase activity pattern best approximates the one found in man.

MATERIALS AND METHODS

Chemicals

NADPH and NADH were from Boehringer (Germany) and HPLC chemicals from Merck (Czech Republic). B was obtained from the

Research Institute of Pharmacy and Biochemistry, Prague (Czech Republic), and B N-oxide was prepared in our laboratory. All other chemicals used were of analytical grade and purchased from Lachema.

Animals and biological material

Male Wistar rats (*Rattus norvegicus var. alba*) (200-250 g), mice (*Mus musculus var. alba*) (20-25 g), guinea-pigs (*Cavia aperea var. porcellus*) (450-500 g) and rabbits (*Oryctolagus cuniculus var. chinchilla*) (about 3 kg) were obtained from Velaz (Prague, Czech Republic). They were fed a standard diet, fasted 12 h prior to the experiment, and sacrificed by decapitation under ether anaesthesia to obtain the liver. Mini-pigs (*Sus scrofa var. alba Göttingen mini-pig*) were obtained from the breeding station of The Military Research Institute (Hradec Králové, Czech Republic). The mini-pigs were sacrificed by bleeding to death under total anaesthesia, their liver removed and used to prepare liver homogenates. Human liver samples were obtained from the Tissue Bank of The Faculty of Medicine, Charles University, Hradec Králové. The microsomal fraction was obtained by fractional ultracentrifugation of the liver homogenate in 0.1 M Na-phosphate buffer (pH 7.4).

Incubation and extraction

The conditions of incubations (substrate and coenzyme concentrations, time of incubation) were chosen according to previous experimental results /3/. The microsomal suspension (0.1 ml in 0.1 M Na-phosphate buffer, pH 7.4, i.e. aliquot containing 1 mg of microsomal proteins) was incubated with 0.5 μmol of substrate and 0.6 μmol of coenzyme NADPH or NADH in 0.3 ml (total volume) of reaction mixture. For inhibition studies cytochrome P450 in microsomal suspension was inhibited prior to incubation (2 min flow of carbon monoxide). Incubations were carried out at 37°C for 30 min under aeration or in argon atmosphere. All incubations were terminated by adding 0.1 ml 13% aqueous ammonia solution. Samples were extracted three times with double their volumes of distilled ethyl acetate and the combined extracts were vacuum-evaporated to dryness.

Protein and P450 content determination

Protein was determined according to Lowry *et al.* with 0.1% SDS /10/. The amount of P450 was estimated using the Omura and Sato method of differential spectrum analysis after CO-reduction /11/.

Chromatographic separation and detection

A Spectra Physics chromatograph was used for HPLC detection, separation, quantification and spectral identification /12/]. The configuration used was as follows: solvent degasser SCM400, quaternary gradient pump P4000, autosampler AS3500 with 100 µl sample loop, Spectra FOCUS high speed scanning UV detector, system controller, computer with analytical software working under OS-2. Analyses were performed on HPLC column LiChroCART 125 x 4 mm I.D, with precolumn LiChroCART 4 x 4 mm I.D., containing LiChrospher 100 RP-18 (5 µm). The mobile phase consisted of 0.01 M nonylamine buffer, pH=7.4, acetonitrile and 2-propanol (2:2:1, v/v/v). The flow rate of the mobile phase was 0.9 ml/min. Detection was performed in dual wavelength mode (295 and 340 nm) and in high-speed scanning mode (range 195-365 nm with 5 nm distance). The determination of B and its metabolites was performed using an external standard method at wavelength 295 nm (for 7-oxo-7*H*-benzo(*c*)-fluorenes) and 340 nm (for 7-hydroxy-7*H*-benzo(*c*)fluorenes), respectively /4/ (Fig. 2).

RESULTS

Microsomes isolated from liver homogenates of various animal species were incubated with B N-oxide (substrate) and NADPH under aerobic conditions. The yields of metabolites generated by reduction I and reduction II are compared in Fig. 3. It is obvious that the ratio of the two reductive pathways varies from species to species. Reduction I accounts for as much as 97% (in the rat) or a mere 18% (rabbit) of the total yield of the metabolites generated by both reductive transformation pathways (= 100%), the yields of pathway I metabolism in the other species studied being 87% (mini-pig), 73% (mouse), 50% (guinea-pig) and 21% (man).

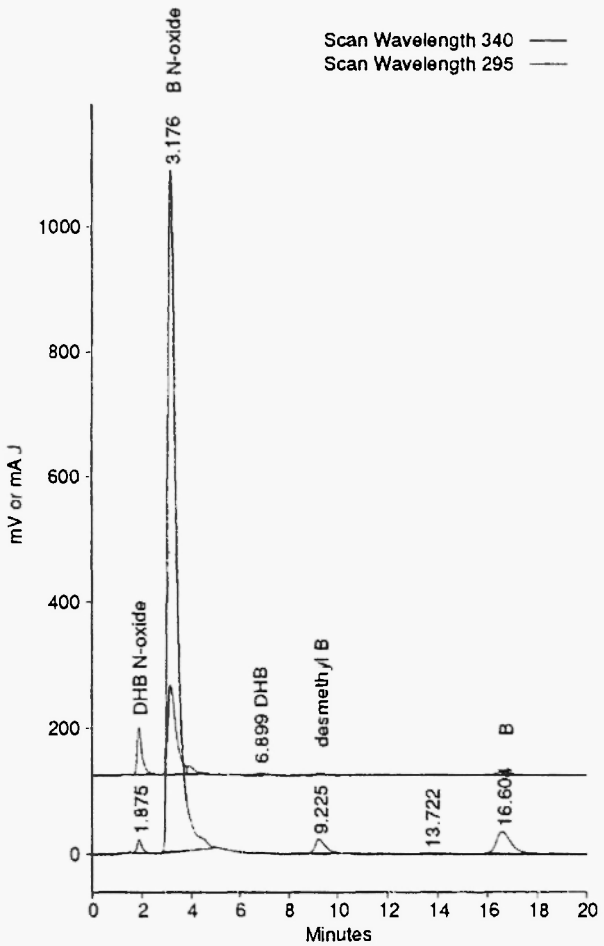

Fig. 2: HPLC chromatogram of B N-oxide and its metabolites. Anaerobic incubation of human microsomes with substrate and NADPH.

Some of the principal properties of microsomal B N-oxide reductases from various species were determined by incubating microsomes with NADPH or NADH under aerobic or anaerobic conditions. The effect of CO, an inhibitor of cytochrome P450, was also examined. The characteristics of the enzymes participating in reduction I and II are given in Figures 4-6.

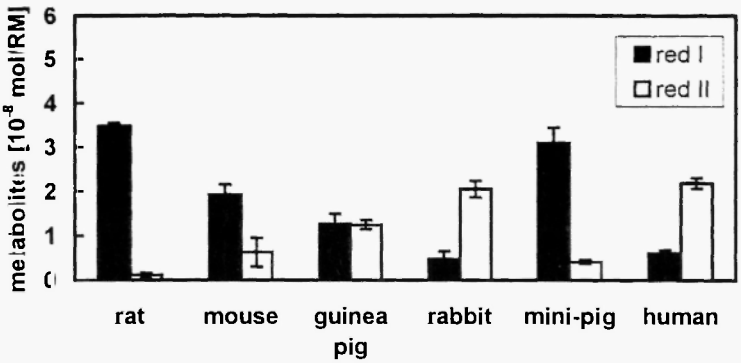

Fig. 3: Inter-species differences in microsomal reductions of B N-oxide. The substrate (B N-oxide) was incubated with NADPH and microsomes under aerobic conditions. The values on the y-axis give the quantities of metabolites in the reaction mixture containing 1 mg microsomal proteins. Average values from 6 samples ± SD.

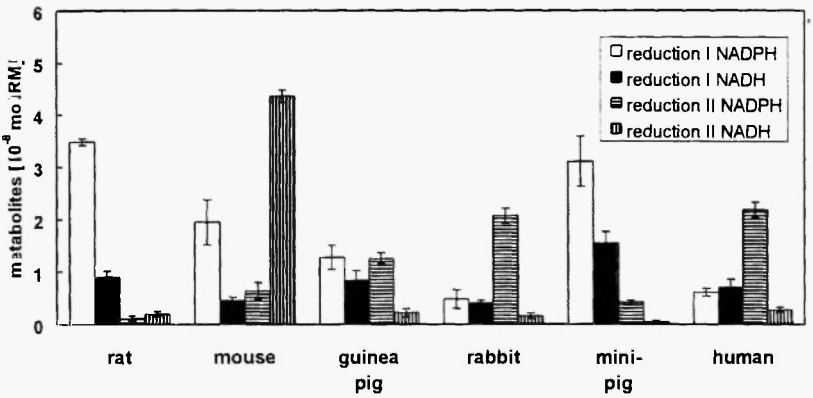

Fig. 4: Inter-species comparison between the effect of NADPH and NADH on B N-oxide microsomal reduction I and II under aerobic conditions. See Fig. 3 for details.

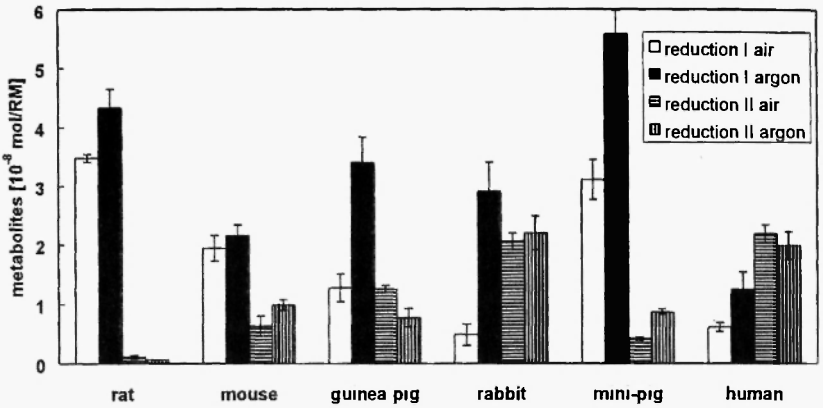

Fig. 5: Inter-species differences in the effect of oxygen on B N-oxide microsomal reduction I and II. The substrate (B N-oxide) was incubated with microsomes and NADPH under aerobic (air) or anaerobic (argon) conditions. See Fig. 3 for details.

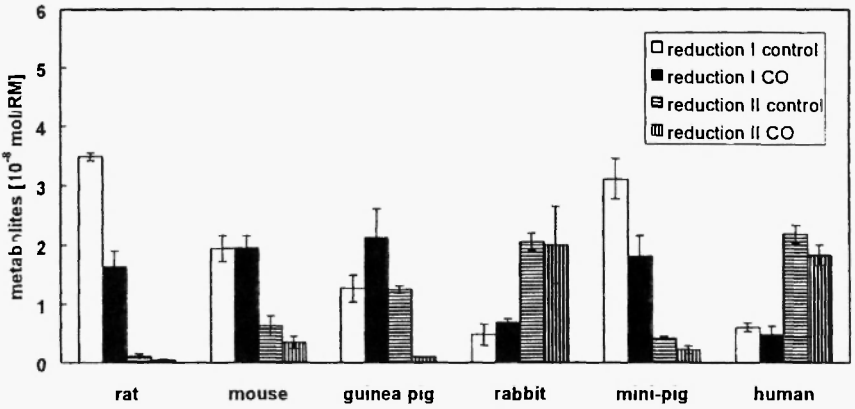

Fig. 6: Inter-species comparison of the effect of CO on microsomal B N-oxide reduction I and II. The substrate (B N-oxide) was incubated with NADPH and microsomes (control or CO inhibited) under aerobic conditions. See Fig. 3 for details.

For the purpose of inter-species comparison of reduction I, based on the preference for NADPH or NADH by the reductases involved, the species studied can be split into two groups. The first group is that in which the reduction I process enzymes prefer NADPH ($p=0.01$): rat, mouse and guinea-pig. The reductases of the other species studied can utilise either co-enzyme (no statistically significant difference). Comparison of the reduction I process studied in various species under aerobic (aeration) or anaerobic (argon atmosphere) conditions shows that anaerobic conditions increased the yield of the reduction I product in all species studied ($p=0.01$) except the mouse. Incubation of B N-oxide with CO-inhibited microsomes from rat and mini-pig significantly reduced the yield of the reduction I product in comparison with controls by 54% and 43%, respectively.

In the reduction II pathway, regarding the preference for NADPH or NADH, mouse reductases catalysing this B N-oxide transformation displayed distinct NADH dependence, unlike the reductases from other species for which NADPH was the preferred coenzyme. Anaerobic conditions increased the yield of the reduction II product by 55% in mouse and by 107% in mini-pig in comparison with aerobic incubations. In guinea-pig microsomes, on the other hand, aerobic conditions increased the yield of the reduction II product by 62%. The most significant inhibitory effect by CO of the reduction II pathway (by 92% in comparison with controls) was observed in the guinea-pig, while only a partial inhibitory effect was observed in mini-pig and mouse (by 45% and 46%, respectively). No significant effects of anaerobic or aerobic conditions or CO were observed in the microsomes from the other species studied.

DISCUSSION

Inter-species comparison of the contribution of the reduction I and reduction II pathways to the reductive transformation of B N-oxide shows that the reduction II pathway - neglected in experiments with rat microsomes up to now - is of minor importance only in the rat and, partly, in the mini-pig. In all the other species studied, however, the metabolic product of the reduction II pathway (DHB N-oxide) represents either a significant (in mouse and guinea-pig) or the major metabolite (in rabbit and man).

The reduction I pathway, which is the main transformation pathway in the rat, was studied in detail in our previous studies /3/. However, inter-species comparison has shown that, in the majority of other species (namely in rabbit and man), the microsomal reductases display significantly lower reduction I activities. Similar inter-species differences have been observed in studies on the N-reduction of N-oxides of some psychotropic substances (chloropromazine N-oxide, amitryptyline N-oxide). In rat, these substances have been found to undergo N-reduction to a greater extent than in man /6/. N-Oxide-structure-dependent differences in the activities of reductases in the rat are documented by only a minimum conversion by rat liver microsomes of tramadol N-oxide to tramadol /13/ while rat microsomes display significant reductase activity towards a number of other N-oxides of tertiary amines /6/.

Cytochrome P450 plays a role in the reduction I pathway /3/. Based on data from the literature /6,14,15/, cytochrome P450 was assumed to play a key role in the transformation of B N-oxide to its parent compound (a tertiary amine) in other species as well as the rat. From this aspect, our results are surprising. Inter-species comparison of preference by the reduction I enzyme(s) for individual coenzymes revealed that NADPH was the preferred coenzyme for the reduction I pathway only in the rat, mouse and mini-pig. A significant inhibitory effect of CO on the reduction I pathway was only observed in rat and mini-pig microsomes, while with mice, rabbit and human microsomes no CO inhibition of reduction I was observed. On the other hand, increased activity of the reduction I pathway in the presence of CO was observed in the case of guinea-pig microsomes; this might be explained by cytochrome P450 participating in the re-oxidation of B to B N-oxide rather than B N-oxide reduction. If this were true, the inhibition of cytochrome P450 would ultimately result in a higher yield of the reduction pathway's product. Anaerobic conditions significantly increased the yield of the reduction I pathway product in all species studied except mice. In addition to the elimination of oxygen's inhibitory effect under anaerobic conditions, this increase may also be a result of blocked re-oxidation of the product B back to B N-oxide. Based on the principal characteristics of the reductases participating in the conversion of B N-oxide to B derived from these experiments, one can assume that cytochromes P450 play a role in the process only in the rat and mini-pig, while participation of another enzyme system

must be assumed in the case of the other species. An interesting finding is that the quantitative contribution of the reduction I pathway is the most significant one in those species in which cytochrome P450 participation in the process is assumed.

The enzymology and mechanisms of the reduction of N-oxides of tertiary amines have been studied extensively in recent years since a number of N-oxides have been shown to undergo selective reduction under anaerobic conditions, and N-oxides of cytotoxic amines are believed to be likely bioreducing agents able to act selectively on hypoxic cells of solid tumours /16-21/. Inter-species differences in the participation of enzymes catalysing certain specific reductive transformation pathways have been observed, e.g. in the case of tirapamazine N-oxide. While NADPH-cytochrome P450 reductase plays the key role in the microsomal reduction of the compound in the mouse /22/, the enzyme accounts for a mere 30% of the reduction pathway in the rat, while 70% of the reduction is catalysed by cytochrome P450 /23/. CO-insensitive reduction of a pyrazine N-oxide has been observed using mouse microsomes that are able to utilise both NADPH and NADH as coenzyme, and participation of NADH-cytochrome b_5 reductase and NADPH-cytochrome P450 reductase has recently been demonstrated /24/. An additional enzyme system which, in addition to cytochrome P450, cytochrome b_5 and their respective reductases, might participate in the reduction of B N-oxide to B is NADH-dependent O_2-insensitive hydroxylamine reductase which catalyses the reduction of a number of amidoximes and probably also N-oxides /25/.

In previous studies we did not investigate the reduction II pathway, which is of minor importance in the rat and which generates the metabolic product at levels on the verge of its detection limits. The present inter-species comparison demonstrates the quantitative importance of this reduction pathway in the biotransformation of B N-oxide in other species. The microsomal reductases catalysing the reduction II pathway display inter-species differences in both their activities and the basic properties studied. The mouse is the only species preferring NADH while NADPH is the preferred coenzyme in all the other species studied. Preference for anaerobic conditions was observed in the guinea-pig, rabbit and man. The significant inhibitory effect of CO on the reduction II pathway in the guinea-pig suggests that cytochrome P450 is involved in the reduction in this species. As

for the other species, other enzyme systems seem to play a major role in the reduction II pathway.

Carbonyl containing xenobiotics can be reduced by both cytoplasmic and microsomal enzymes. Enzymology of the reductions is rather unclear /6/. Cytochrome P450 and its reductase play an important role in the reduction of the carbonyl group of quinones and semiquinones. Due to its single-electron mechanism and concomitant generation of free radicals, the reaction is of considerable toxicological importance in the metabolism of a number of xenobiotics including some drugs (adriamycine, mytomycine, daunorubicine) /15,26/. An additional well-characterised enzyme system is 11-β-hydroxysteroid dehydrogenase. This enzyme has been isolated from mouse microsomes /27/ and demonstrated to be able to reduce metyrapone /28/, nitrosamines /29/ and a number of additional xenobiotics, and to use both NADH and NADPH as coenzyme /30/. The microsomal reductase of S-warfarin has been shown to have interesting properties. This NADPH-dependent, highly stereospecific enzyme has also been shown to display sex differences /31/. Another reductase activity with sex-specific differences has been observed in the case of microsomal acetohexamide reductase /32,33/. Various inter-species comparisons of reductases have revealed significant similarities of metyrapone carboxyl reductase from mouse, rat and guinea-pig /34/, and from mouse and man /35/.

Our experiments demonstrated significant inter-species differences in microsomal carbonyl reductase activity displayed with B N-oxide as substrate, the differences being observed in all properties investigated. On the other hand, B N-oxide reductase from rabbit and man displayed considerable similarities. Also of importance is the finding that rabbit and human microsomes are virtually identical concerning both the reduction II and reduction I pathways, the similarity being not just in the quantitative pattern of the reductases but also in all their properties examined. On the other hand, as far as the enzyme systems involved in the oxidative transformation pathway of B are concerned, the highest degree of similarity was observed between human and mini-pig microsomes /36/. In comparison with human microsomes, however, mini-pig microsomes displayed quite different properties with respect to the reductive transformation of B N-oxide. Therefore, the mini-pig cannot be considered a suitable species for comprehensive studies on B N-oxide biotransformation and, probably, of benfluron or its derivatives

either. Our experiments clearly demonstrate that the rabbit is the optimum model species to study reductive transformation of B N-oxide for extrapolation to man.

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REFERENCES

1. Kvasnicková E, Nobilis M, Hais IM. Chromatographic characterization of in vitro metabolites of 5[2-(N,N'-dimethylamino)ethoxy]-7-oxo-7H-benzo[*c*]-fluorene. J Chromatogr 1984; 295: 201-209.
2. Kvasnickova E, Hais IM, Nobilis M. Relationship between NADPH and NADPH as cosubstrates of N-oxidation of the cytostatic benfluron by FMO. Prog Pharmacol Clin Toxicol 1991; 7: 146.
3. Skálová L, Nobilis M, Szotaková B, Trejtnar F, Kvasnickova E. In vitro and in vivo biotransformation of benfluron N-oxide, a biologically active metabolite of a potential cytostatic. Exp Toxic Pathol 1996; 48: 366-370.
4. Nobilis M, Kvasničková E, Sroler A, Hais IM. Elimination of benfluron and its metabolites in the faeces and urine of rats. Drug Metab Drug Interact 1991; 9: 225-240.
5. Vančurová I et al. Patent application no. PCT/CS88/239413.
6. Testa B. Reduction catalyzed by cytochrome P450 and other oxidoreductases. In: Testa B, Caldwell J, eds. The Metabolism of Drugs and Other Xenobiotics. Biochemistry of Redox Reactions. London: Academic Press, 1995; 398-447.
7. Benakis A, Halpaap B. Reductive metabolism and toxicological implications. Exp Toxic Pathol 1996; 48: 25-33.
8. Fink-Gremmels J, Van Miert AS. Species differences in biotransformation. Conventional models in unconventional animal species. Exp Toxic Pathol 1996; 48: 120-127.
9. Valles B, Schiller CD, Coassolo P, De Sousa G, Rahmani R. Metabolism of mofarotene in hepatocytes and liver microsomes from different species. Drug Metab Dispos 1995; 23: 1051-1057.
10. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin C phenol reagent. J Biol Chem 1951; 193: 165-275.
11. Omura T, Sato R. The carbon-monoxide-binding pigment of liver microsomes. II. Solubilization, purification, properties. J Biol Chem 1964; 239: 2379-2385.
12. Nobilis M, Anzenbacher P, Pastera J, Svoboda Z, Hruby K, Kvitina J, Ubik K, Trejtnar F. Study of biotransformation of a potential benzo[*c*]fluorene antineoplastic using HPLC with high-speed-scanning UV detection. J Chromatogr B 1996; 681: 143-151.

13. Raffa R, Haslego ML, Maıyanoff CA, Villani FJ, Codd EE, Connelly CD, Martinez RP, Schupsky JJ, Buben JA, Wu VN, Takacs AN, Mckown LA. Unexpected antinociceptive effect of the N-oxide (RWJ 38705) of tramadol hydrochloride. J Pharmacol Exp Ther 1996; 278: 1098-1104.
14. Wislocki PG, Miwa GT. Lu AYH. Reactions catalyzed by cytochrome P450 system. In: Jakoby WB, ed. Enzymatic Basis of Detoxication. London: Academic Press, 1980.
15. Goeptar RA, Scheerens H, Vermeulen NPE. Oxygen and xenobiotic reductase activities of cytochrome P450. Crit Rev Toxicol 1995; 25: 25-65.
16. Patterson AV, Barham HM, Chinje EC, Adams GE, Harris AL, Stratford IJ. Importance of P450 reductase activity in determining sensitivity of breast tumour cells to the bioreductive drug, tirapazamine (SR 4233). Br J Cancer 1995; 72: 1144-1150.
17. Elwell JH, Siim BG, Evans JW, Brown JM. Adaptation of human tumor cells to tirapazamine under aerobic conditions. Biochem Pharmacol 1997; 54: 249-257.
18. Tercel M,Wilson WR, Denny WA. Hypoxia-selective antitumor agents. 11. Chlorambucil N-oxide: a reappraisal of its synthesis, stability, and selective toxicity for hypoxic cells. J Med Chem 1995; 38: 1247-1252.
19. Patterson LH. Rationale for the use of aliphatic N-oxides of cytotoxic anthraquinones as prodrug DNA binding agents: a new class of bioreductive agent. Cancer Metastasis Rev 1993; 12: 119-134.
20. Riley RJ, Workman P. Enzymology of the reduction of the potent enzotriazine-di-N-oxide hypoxic cell cytotoxin SR 4233 (WIN 59075) by NAD(P)H: (Quinone acceptor) oxidoreductase (EC 1.6.99.2) purified from Walker 256 rat tumour cells. Biochem Pharmacol 1992; 43: 167-174.
21. Wilson WR, Van Zijl P, Denny WA. Bis-bioreductive agents as hypoxia-selective cytotoxins: nitracrine N-oxide. Int J Radiat Oncol Biol Phys 1992; 22: 693-696.
22. Fitzsimmons SA, Lewis AD, Riley RJ, Workman P. Reduction of 3-amino-1,2,4-benzotriazine-1,4-di-N-oxide (tirapazamine, WIN59075, SR4233) to a DNA-damaging species: a direct role for NADPH: cytochrome P450 oxidoreductase. Carcinogenesis 1994; 15: 1503-1510.
23. Walton MI, Wolf CR, Workman P. The role of cytochrome P450 and cytochrome P450 reductase in the reductive bioactivation of the novel benzotriazine di-N-oxide hypoxic cytotoxin 3-amino-1,2,4-benzotriazine-1,4-dioxide (SR 4233, WIN 59075) by mouse liver. Biochem Pharmacol 1992; 44: 251-259.
24. Barham HM, Stratford IJ. Enzymology of the reduction of the novel fused pyrazine mono-N-oxide bioreductive drug, RB90740: roles for P450 reductase and cytochrome b_5 reductase. Biochem Pharmacol 1996; 51: 829-837.
25. Clement B, Lomb R, Moller W. Isolation and characterization of the protein components of the liver microsomal O_2-insensitive NADH-benzamidoxime reductase. J Biol Chem 1997; 272: 19615-19620.
26. Pröpper D, Maser E. Carbonyl reduction of daunorubicin in rabbit liver and heart. Pharmacol Toxicol 1997; 80: 240-245.

27. Maser E, Bannenberg G. The purification of 11β-hydroxysteroid dehydrogenase from mouse liver microsomes. J Steroid Biochem Mol Biol 1994; 48: 257-263.
28. Rekka EA, Soldan M, Belai I, Netter KJ, Maser E. Biotransformation and detoxification of insecticidal metyrapone analogues by carbonyl reduction in the human liver. Xenobiotica 1996; 26: 1221-1229.
29. Maser E, Richter E, Friebertshäuser J. The identification of 11-beta-hydroxysteroid dehydrogenase as carbonyl reductase of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Eur J Biochem 1996; 238: 484-489.
30. Maser E, Bannenberg G. 11β-Hydroxysteroid dehydrogenase mediates reductive metabolism of xenobiotic carbonyl compounds. Biochem Pharmacol 1994; 47: 1805-1812.
31. Apanovitsch D, Walz FG Jr. Adult-male-specific S-warfarin (11S-OH) and progesterone (20β-OH) keto-reductases in rat hepatic microsomes are not identical. Biochim Biophys Acta 1996; 1291: 16-26.
32. Imamura Y, Kimura R, Kozono Y, Otagiri M. Strain- and sex-related differences of acetohexamide reductase activities in liver microsomes and cytosol of rats: The Wistar-Imamichi strain lacks the male-specific microsomal enzyme activity. Life Sci 1995; 57: 1811-1817.
33. Imamura Y, Takada H, Kimura R, Mori Y, Otagiri M. Inheritance of aceto-hexamide reductase activities in liver microsomes and cytosol of rats. Life Sci 1996; 59: 2201-2208.
34. Oppermann UC, Maser E, Mangoura SA, Netter KJ. Heterogeneity of carbonyl reduction in subcellular fractions and different organs in rodents. Biochem Pharmacol 1991; 42 (Suppl): 189-195.
35. Maser E, Hahnemann B, Legrum W, Oppermann U, Netter KJ. The influence of cytochrome P-450 inducers on carbonyl reduction in mouse liver. Arch Toxicol 1991; 14 (Suppl): 188-192.
36. Anzenbacher P, Soucek P, Gut I, Hruby K, Anzenbacherová E, Kvetina J. Cytochrome PATO3A, the most important enzyme of drug metabolism in human liver, is present in minipig liver microsomes in similar content and activity to human samples. ISSX Proc 1997; 11: 231.